AMENDMENTS TO THE SPECIFICATION:

In compliance with 37 C.F.R. § 1.823(a), please replace the Sequence Listing originally filed with the attached paper copy of a substitute Sequence Listing after the last page of the above-identified application.

Please replace paragraph [0018] at page 7, with the following amended paragraph:

[0018] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

Fig. 1 is a schematic diagram illustrating the principles of the sandwich ELISA and the open sandwich ELISA.

Fig. 2 is a schematic diagram illustrating the principle of the phage display method.

Fig. 3 is a schematic diagram illustrating the principles of the method for displaying a fragment of an antibody in the form of a single chain and the method for displaying the VH fragment and the VL fragment separately.

Fig. 4 is a schematic diagram illustrating the split Fv-coding region of the phagemid vector Pks ((Gly_4Ser) linker SEQ ID NO:40 & 6-His tag SEQ ID NO:41).

Fig. 5 is a graph illustrating the result of investigation on antibody display by ELISA.

Fig. 6 is a graph illustrating the result of investigation on the antigen binding activity by ELISA.

Fig. 7 is a graph illustrating the result of the investigation on antigen (HEL) binding activity by ELISA with respect to scFv type and split Fv type phages displaying HyHEL-10 at phage concentration of 1010 cfu/ml.

Fig. 8 is a graph illustrating the result of investigation on the antigen (HEL) binding activity by ELISA with respect to scFv type and split Fv type phages displaying HyHEL-10 at phage concentration of 109 cfu/ml.

Fig. 9 is graphs illustrating the results of open sandwich ELISA with respect to pKS1(HyHEL10)/sup+.

Fig. 10 is a schematic diagram illustrating the mechanism underlying the display switch in the experimental system according to invention.

Fig. 11 is a graph illustrating the result of open sandwich ELISA using HyHEL10 and D1.3.

Fig. 12 is a graph illustrating the ratio of positive clones accompanied with panning operation.

Fig. 13 is a schematic diagram illustrating the process for preparation of HyHEL-10 or D1.3 type split Fv fragments (SEQ ID NOS:27-33 AND SEQ ID NOS:34-39).

Fig. 14 is a schematic diagram illustrating the process from preparation of phage library to selection of clones with high HEL binding ability.

Fig. 15 is a figure showing the relationship between HEL binding ability and suitability for open sandwich ELISA.

Fig. 16 is a figure showing relationship between HEL binding activity and VH/VL interaction

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Please replace paragraph [0042] at page 17, with the following amended paragraph:

[0042] Cloning of ompA-FLAG

The genes encoding E.coli ompA secretion signal sequence and FLAG tag sequence located at the N-terminal of VL were amplified by PCR using a plasmid pFLAG-ATS (Sigma-Aldrich) as a template. The primer sequences were designed such that the restriction sites of the restriction enzymes XbaI and SalI were inserted at the 5'-side and 3'-side of the amplified fragment, respectively. The amplified DNA was cloned into a cloning vector pHSG397 (TakaraBio, Otsu, Japan), and its sequence was confirmed using a fluorescent DNA sequencer (SQ-5500, Hitachi, Tokyo) and Thermosequenase sequencing kit (Amersham Bioscience, Tokyo)(this cloned vector is called pHSG397/ompA-FLAG hereinafter).

Back: ompXbaRV (SEQ ID NO:3)

5'-CGGGGTCGACTGTGCACTTTTGTCATCGTCGTCCTTGTAG-3'

Forward: ompApaSalFR (SEQ ID NO:4)

5'-CGGGGTCGACTGTGCACTTTTGTCATCGTCGTCCTTGTAG-3'

Please replace paragraph [0043] at page 17, with the following amended paragraph:

[0043] Cloning of gene IX, gene VII

A single strand DNA (ssDNA) was prepared from a helper phage M13KO7 (TakaraBio), and the sequences of gene VII and gene IX were amplified by PCR using the ssDNA as a template. The primers used were as follows and they are designed for the purpose to introduce KpnI and EcoRI sites for gene VII, XhoI and XbaI sites for gene IX at the 5'- and 3'-termini respectively, as the restriction sites of restriction enzymes.

Back: g7KpnRV (SEQ ID NO:5)

5'-CGGGGGTACCGCAGGTCGCGGATTTCGAC-3'

Forward: g7EcoFR (SEQ ID NO:6)

5'-CGGGGAATTCTCATCTTTGACCCCCAGCG-3'

Back: g9XhoRV (SEQ ID NO:7)

5'-CGGGCTCGAGCGGTGGAGGCGGTTCAATGAGTGTTTTAGTGTATTCTTTC-3'

Forward: g9XbaFR (SEQ ID NO:8)

5'-CGGGTCTAGATCATGAGGAAGTTTCCATTAAAC-3'

Please replace paragraph [0046] at page 18, with the following amended paragraph:

[0046] The VH, VL and linker were amplified respectively by PCR using the following primers, and then each fragment of VH-linker and linker-VL were amplified by overlap-extension PCR. Further, by performing overlap-extension PCR on the VH-linker and linker-VL, a fragment of VH-linker-VL was obtained. Both termini of the fragment were digested with restriction enzymes NcoI and NotI.

PCR amplification of linker:

Back: LinkBackX (SEQ ID NO:9)

5'-GGGACCACGGTCACCGTCTCGAGCGGTGGAGGCGGTTCAATG-3'

Forward: LinkFor (SEQ ID NO:10)

5'-AGACTGGGTGAGCTCAATGTCCGTCGACTGTGCACTTTTGTC-3'

PCR amplification of VH (HyHEL-10):

Back: M13RV (SEQ ID NO:11)

5'-CAGGAAACAGCTATGAC-3'

Forward: VH1For2X (SEQ ID NO:12)

5'-GACGGTGACCGTGGTCCCTTGGCCCC-3'

PCR amplification of VL (HyHEL-10)

Back: Vk2Back (SEQ ID NO:13)

5'-GACATTGAGCTCACCCAGTCTCCA-3'

Forward: ReverseSEQ (SEQ ID NO:14)

Reference, 5'-GTAAAACGACGGCCAGT-3'

Please replace paragraph [0047] at page 18, with the following amended paragraph:

[0047] Construction of phagemid vector pKS1

A phagemid vector pKS1 was constructed on pScFv3, which is a modified version of pK1 (Kristensen, P., and Winter, G. (1998) Folding & Design 3:321-328). PCR was performed using phagemid vector pIT2(13CG2) (de Wildt, R.M., Mundy, C.R., Gorick, B.D., and Tomlinson, I.M. (2000) Nat. Biotechnol., 18:989-994) encoding anti bovine serum albumin (BSA) single chain antibody (scFv) as a template with primer M13RV and MycAKpnFor (5'-CCGGGTACCTATGCGGCCCCATTCAGATC-3') (SEQ ID NO:15), to amplify DNA fragments encoding anti BSA scFv and His-Myc tag. The DNA fragment, after dijested with SfiI, received end blunting with T4 DNA polymerase, then it was treated with KpnI and purified.

Please replace paragraph [0065] at page 24, with the following amended paragraph:

[0065] A phagemid vector pKS2 was digested at SapI site upstream of Lac promoter. The following four oligonucleotides were phosphorlylated and annealed to prepare a terminator gene, which was inserted into the cleaved site.

tHP1 5'-AGC GGT ACC CGA TAA AAG CGG CTT CCT GAC-3' (SEQ ID NO:16)

tHP2 5'-AGG AGG CCG TTT TGT TTT GCA GCC CAC CTC-3' (SEQ ID NO:17)

tHP3 5'-GCT GAG GTG GGC TGC AAA ACA AAA CGG CCT-3' (SEQ ID NO:18)

tHP4 5'-CCT GTC AGG AAG CCG CTT TTA TCG GGT ACC-3' (SEQ ID NO:19)

Please replace paragraph [0067] at page 24, with the following amended paragraph:

[0067] Then, a phagemid pKS2T was digested at an EcoRI site existing downstream of the fusion protein gene. The following two primers tHP7 and tHP8, as well as tHP2 and tHP3 were annealed to prepare a terminator gene, which was inserted into the cleaved site.

tHP7 5'-AAT TGG TAC CCG ATA AAA GCG GCT TCC TGA C-3' (SEQ ID NO:20)

tHP8 5'-AAT TGA GGT GGG CTG CAA AAC AAA ACG GCC T-3' (SEQ ID NO:21)

Please replace paragraph [0077] at page 27, with the following amended paragraph:

[0077] Next, VH FR2, VL FR2 and linker (FR2) were assembled into one DNA fragment by overlap extension PCR. First, in the absence of primers, VH FR2, VL FR2 and linker (FR2) were added into a PCR solution, and reaction started from (94°C, 5 min), then 7 cycles of (94°C, 30 sec; 55°C, 30 sec; 72°C, 1 min), and ended at 4°C. Then, using MH2BackSfi as a reverse primer and g7EcoFR as a forward

primer, reaction was performed 30 cycles of (94°C, 30 sec; 55°C, 30 sec; 72°C, 1 min), and ended at 4°C. The used polymerase was Ex Taq.

H10VHframe2:

5'-

ACCACTGTAGCTTACGTACCCCAWSYACTCCAGACSKTTACCTGGARRTTKACG

AAYCCAGCTCCAATAATCACTGGT-3' (SEQ ID NO:22)

H10VLframe2:

5'-

GGCAACAACCTACACTGGTATCAACAAAAAYMGSRCRAATCTCCTCRGCTCCTG

RTCWAKTATGCTTCCCAGTCCATCTCT-3' (SEQ ID NO:23)

H10linkRV:

5'-GGGTACGTAAGCTACAGTG-3' (SEQ ID NO:24)

H10linkFR:

5'-GATACCAGTGTAGGTTG-3' (SEQ ID NO:25)

(K=G or T, M=A or C, R=A or G, Y=C or T, S=C or G, W=A or T)

Please replace paragraph [0078] page 28, with the following amended paragraph:

[0078] Confirmation of the randomization of FR2 region

The split Fv fragment thus amplified was digested with NcoI and NotI, inserted into a vector pKST2 treated with NcoI and NotI. The resulting vector was used to transform E.coli TG-1sup+. The cells were applied to a YTAG plate (Ap, 100 μg/mL; glucose, 1%), and the plate was incubated at 30°C overnight. Colony PCR was performed on the resultant colonies. The insertion of split Fv fragment was

confirmed on the clones, such clones were inoculated from a replica plate, and the culture was incubated at 37°C overnight with shaking. Plasmid was purified from the resultant bacterial culture by alkaline SDS method. The sequence was confirmed with an ABI PRISM 3100 genetic analyzer (Applied Biosystems) and BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). The used primers were M13RV, and OmpARV (reverse primer complimentary to the partial sequence of OmpA signal). To confirm phage display of VH and VL, then to confirm the display of functional Fv, anti-FLAG M2, 9E10 and antigen HEL, were immobilized respectively and phage ELISA was performed along with blank wells. Fig. 13 shows a schematic diagram showing production of split Fv fragments wherein respective residues in FR2 region of HyHEL-10 are modified to HyHEL-10 type or D1.3 type.

OmpARV:

5'-ACAGCTATCGCGATTGCAGTG-3' (SEQ ID NO:26)